(19) JAPANESE PATENT OFFICE (JP)

(12) **Official Gazette for Laid-Open Patent Applications (A)**

(11) Japanese Laid-Open Patent Application (Kokai) No. **5-320024**

(51) Int.Cl.5		Ident. Symbols	Internal Office Nos. FI
A61K	7/00	X	9165-4C
		K	9165-4C
	7/48		9051-4C

(43) Laying-Open Date: 3 December 1993

Request for Examination: Not yet requested

Number of Claims: 1 (Total of 4 pages)

(21) Application No.: 4-154414

(22) Application Date: 21 May 1992

(71) Applicant: 000224798
Dowa Mining Company, Ltd.
8-2 Marunouchi 1-chome, Chiyoda-ku
Tokyo-to

(72) Inventor: Masayuki Suzuki
c/o Dowa Mining Company, Ltd.
8-2 Marunouchi 1-chome, Chiyoda-ku
Tokyo-to

(72) Inventor: Kazunari Shigematsu
c/o Dowa Mining Company, Ltd.
8-2 Marunouchi 1-chome, Chiyoda-ku
Tokyo-to

(72) Inventor: Yu-Pan Chen
c/o Hsin-An Research Laboratories
No. 116, Section 3, Chung Ching South Road
Taipei City, Taiwan

(74) Agent: Masahiko Maruoka, Patent Attorney

Continued on last page

(54) **[Title of the Invention] A Beautifying and Whitening Cosmetic Material**

(57) [Abstract]

[Objective] To provide a beautifying and whitening cosmetic material which has a superior whitening effect on the skin and which has sufficient storage stability and high safety.

[Structure] First, approximately 100 g of dried mulberry cuttings, which is a crude drug, is pulverized in a mixer, the pulverized matter and 1 liter of 50% ethyl alcohol are introduced into a flask and refluxing and extraction are performed at 50°C as the materials are being stirred. After extraction, this solution is suctioned and filtered and the filtrate that is obtained is concentrated at 50°C using an evaporator. Next, the concentrated solution that is obtained is dried under reduced pressure and 9.2 g of brown crystals (extracted matter) is obtained. The extracted matter that is obtained is compounded as the effective component with 0.01 to 5.0% of cosmetic material.

[Claims]

[Claim 1] A beautifying and whitening cosmetic material characterized in that an extracted material of at least one crude drug is selected from a group comprised of mulberry cuttings, black soybeans, achene of Siberian cocklebur [Xanthium sibiricum] and Milletia reticulata.

[Detailed Description of the Invention]

[0001]

[Field of industrial use] This invention relates to a beautifying and whitening cosmetic material having a skin whitening effect. In further detail, it relates to a beautifying and whitening cosmetic material, a crude drug extract, having a whitening effect based on a melanin production inhibiting action as the effective component.

[0002]

[Prior art] It is generally thought that black color, spots and freckles due to sunburn are produced by production of melanin, which is a blackish-brown amorphous pigment. When the skin is subjected to the external irritation of ultraviolet rays, tyrosinase (a tyrosine oxidizing enzyme), which is present in the melanin cells of the skin, is activated and tyrosine, which is a structural amino acid of proteins, is oxidized, with melanin being produced. Consequently, a skin whitening effect can be anticipated by inhibiting the activity of tyrosinase, which is involved in melanin production. For this reason, compounding of a tyrosinase activity inhibiting component with a cosmetic material has been proposed.

[0003] Known beautifying and whitening cosmetic materials having a beautifying and whitening effect include substances obtained by compounding of ascorbic acid or derivatives thereof as disclosed in Japanese Patent Announcement 55-43443 [1980], "A Beautifying and Whitening Cosmetic Material," and in Japanese Patent Announcement 54-974 [1979], "A Crude Drug Extract Compounding Composition." Other known substances having beautifying and whitening effects include topical skin agents in which arbutin is compounded (Japanese Patent Application Early Disclosure No. 60-16906 [1985]), bleaching cosmetic materials in which kojic acid is compounded (Japanese Patent Announcement 32-8100 [1957])

and cosmetic materials extracted from plant components (Japanese Patent Application Early Disclosure No. 63-2913 [1988]) and animal components (Japanese Patent Application Early Disclosure No. 63-8312 [1988]).

[0004] However, many of the aforementioned conventional cosmetic materials do not have a sufficient beautifying and whitening effect. In addition, many of them do not have sufficient storage stability and exhibit safety problems with respect to the skin such as exhibiting irritating effects.

[0005]

[Problems the invention is intended to solve] This invention has the objective of solving the problems of the aforementioned conventional technologies and of providing a beautifying and whitening cosmetic material having a beautifying whitening effect and having sufficient storage stability and high safety.

[0006]

[Means for solving the problems] The inventors conducted intensive research for the purpose of solving these problems. As a result, we discovered that extracts of raw drugs such as mulberry cuttings, black soybeans, achene of Siberian cocklebur [Xanthium sibiricum] and Milletia reticulata have a tyrosinase enzyme activity inhibiting action and a melanin production inhibiting action in melanoma cells.

[0007] Specifically, this invention provides a beautifying whitening cosmetic material characterized in that an extracted material of at least one crude drug is selected from a group comprised of mulberry cuttings, black soybeans, achene of Siberian cocklebur [Xanthium sibiricum] and Milletia reticulata.

[0008]

[Action] The cosmetic material of this invention can be manufactured by the following method. First, a dried material or pulverized fried material consisting of at least one of the following, mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata is heated and extracted using an extraction solvent. Said extraction solvent can be an alcohol (methanol, ethanol, propanol or isopropanol) or water. In addition, mixed solutions of these substances can also be used. For example, when an aqueous solution of alcohol, with an alcohol concentration of 20 to 70% is used and extraction is performed for 1 hour at 50°C, there is excellent extraction.

[0009] After extraction, the extraction solution is separated by filtration and an extraction extract [sic] is obtained. The extraction extract is then concentrated and dried under reduced pressure as it is being heated at a temperature of under 60°C, the dried extract is recovered and is compounded with a cosmetic material. The aforementioned extraction extract may also be compounded with a cosmetic material in unaltered form.

[0010] The inventors confirmed that the extract of the crude drug (mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata) that has been obtained in this way exhibits a superior melanin production inhibiting action at lower concentrations than ascorbic acid, which is used conventionally. A whitening cosmetic material having a beautifying whitening effect can be obtained by compounding this extract as the effective component in amounts of 0.01 to 5.0%.

[0011] We shall now present a more detailed description of this invention by means of examples. However, the scope of this invention is not limited by the following examples.

[0012]

[Example 1] This example illustrates one of the methods of extraction of crude drug. First, approximately 100 g of dried mulberry cuttings, which was the crude drug, was pulverized with a mixer, the pulverized material and 500 ml of 50% ethyl alcohol were introduced into a flask, and refluxing and extraction were performed for 1 hour at 50°C as the materials were being stirred. After extraction, this solution was filtered by suction and the filtrate that was obtained was concentrated under reduced pressure at 50°C using an evaporator. Next, the concentrated solution that was obtained was dried under reduced pressure and 9.2 g of brown crystals (extract) was obtained.

[0013] In addition, 15.6 g, 8.8 g and 11.9 g of extracts were obtained, respectively, in the same way as described above from amounts of approximately 100 g of black soybeans, achene of Siberian cocklebur and Milletia reticulata, which were the crude drugs.

[0014]

[Example 2] In this example, determinations were made of the tyrosinase activity inhibiting action of the extracts of mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata that were obtained in Example 1. Determination of the tyrosinase inhibiting activity was obtained using a method in which dopachrome [sic] produced by tyrosinase from dopa was determined quantitatively by measuring the absorbance at 475 nm. The following reaction reagents were used in the determinations of tyrosinase activity inhibiting action.

(a) Sodium succinate buffer (100 mM, pH 5.5)

(b) Mushroom tyrosinase (manufactured by Sigma Company) solution (prepared to 270 units/ml with buffer (a))

(c) L-dopa (manufactured by Wako Junyaku Co. (Ltd.)) solution (prepared to 5 mM with buffer (a))

First, 1.8 ml of buffer (a) and 0.1 ml of tyrosinase solution (b) were introduced into a test tube, 0.1 ml of a test material solution of a concentration of 2% (w/v) (aqueous solution of the extract obtained in Example 1) was added to this test tube and

the materials were incubated for 15 minutes in a constant temperature water tank at 30°C. Next, 1 ml of L-dopa solution (c) was added to the test tube and the mixture was stirred, after which said test tube was set at an inclination of approximately 45°in a reciprocating vibrator in a constant temperature chamber at 30°C and it was shaken for 40 minutes (reciprocating frequency of 150/minute). After shaking, absorbance was determined at 475 nm using a spectrophotometer and the determined value was designated as A.

[0015] As a control, the same procedure as described above was carried out except that the buffer (a) was added instead of the test material solution. Absorbance was determined at 475 nm and the determined value was designated as B. In addition, as a blank, the same procedure as described above was performed except that buffer (a) was added instead of the L-dopa solution. Absorbance was determined at 475 nm and the determined value was designated as C.

[0016] The tyrosinase activity inhibition rates of the test material solutions were calculated from the aforementioned determined values of absorbance at 475 nm. The calculations of tyrosinase activity inhibition rates were performed using the following equation. The results are shown in Table 1.

[0017] Tyrosinase activity inhibition rate (%) =
$\{1 - (A - C)/B\} \times 100$

[0018]

[Table 1]

Tyrosinase activity inhibitory action	
2% (w/v) aqueous solution of crude drug extract	Tyrosinase activity inhibition rate (%)
Mulberry cuttings	71
Black soybeans	72
Achene of Siberian cocklebur	69
Milletia reticulata	96

[0019] As can be seen from Table 1, extracts of mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata strongly inhibited tyrosinase activity even at the low concentration of 2% (w/v) aqueous solutions. It was confirmed that they have superior tyrosinase activity inhibiting action.

[0020]

[Example 3] In this example, determinations were made of the melanin production inhibitory action of the extracts of mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata that were obtained in Example 1.

[0021] First, B16 melanoma cells (B16-F0, ATCC No. CRL-6322), which are malignant melanoma cells that produce melanin and that originate from mice were cultured in Eagle's MEM culture medium to which bovine fetal serum had been added to give a final concentration of 10%. Amounts of 6 ml of the aforementioned culture medium containing said cells in a concentration of 3 x 10^3 cells/ml were introduced into each well of a 6-well plate (manufactured by the Falcon Company) and the materials were cultured for 5 days in a CO_2 incubator (5% CO_2, 37°C).

[0022] This culture medium was replaced with fresh Eagle's MEM culture medium (6 ml) containing 0.03% theophylline (manufactured by the Sigma Company and a suitable quantity of test material solution (aqueous solution of the extract obtained in Example 1) was added to each well, after which the materials were cultured for an additional 3 days. After culturing was completed, said culture medium was discarded, 1 ml of physiological saline solution was added to each well and the cells that were attached to the bottom faces of the wells were scraped off using a scraper and were suspended in the solution. Next, said cell suspension was transferred using a pipette to a microcentrifuge tube (1.5 ml capacity, manufactured by the Eppendorf Company) and it was centrifuged (1000 x g, 15 minutes).

[0023] As a control, a sterilized solution was added in place of the test material solution and the same experiment as described above was carried out. Further, as an experimental group for testing whitening of cells, amounts of (a) 60 μl, (b) 150 μl and (c) 300 μl of 2% aqueous solution of L-ascorbic acid were added in place of the test material solution and the same experiment as described above was carried out.

[0024] Next, the degree of whitening of cells that had been converted into pellets was compared visually and evaluations of melanin production inhibiting effect were made. The degree of whiteness of the cells in the control experimental group (group in which sterilized water was added) was taken as "-" and the degree of whiteness of the cells in the comparative experimental groups to which L-ascorbic acid had been added were taken, respectively, as (a): "+," (b): "++" and (c): "+++." The degree of whiteness of cells when test material solution was added was evaluated visually in correspondence to -, +, ++ and +++, with the strength of the melanin production inhibitory effect of the test material solution thus being evaluated in 4 levels. The results are shown in Table 2.

[0025]

[Table 2]

Melanin production inhibitory action			
Concentration (μg/ml)	50	200	800
Mulberry cuttings extract	+	+	++
Black soybeans extract	-	+	+
Achene of Siberian cocklebur extract	+	+	++
Milletia reticulata extract	+	+	++
Concentration (μg/ml)	200	500	1000
L-ascorbic acid	+	++	+++

[0026] As can be seen from Table 2, it was confirmed that extracts of mulberry cuttings, achene of Siberian cocklebur and Milletia reticulata exhibited melanin production inhibitory activity equal to that of 200 μg/ml of L-ascorbic acid at concentrations of 50 μg/ml and that they inhibited melanin production at lower concentrations than L-ascorbic acid. The extract of black soybeans exhibited a melanin production inhibitory action equal to that of ascorbic acid in a concentration of 200 μg/ml. Moreover, the extracts were not toxic to cells even at high concentrations of 800 μg/ml.

[0027]

[Example 4] Here, an example is shown of compounding of an extract of mulberry cutting, which was the crude drug, obtained in Example 1 with a cosmetic material.

[0028]

	(wt %)
Mulberry cutting extract	1.0
Glycerol	5.0
Polyoxyethylene sorbitan monolaurate	1.5
Ethanol	10.0
Fragrance	Suitable quantity
Preservative, antioxidant	Suitable quantity
Pigment	Suitable quantity
Purified water	Remainder

[0029]

[Effect of the Invention] Extracts of mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata have superior tyrosinase activity inhibitory action and melanin production inhibitory action in melanoma cells. Beautifying and whitening cosmetic agents of this invention in which at least one of these extracts is compounded exhibit superior skin whitening effectiveness. Further, extracts of mulberry cuttings, black soybeans, achene of Siberian cocklebur and Milletia reticulata that are compounded with amounts of the whitening cosmetic of this invention exhibit superior whitening effects in small quantities and are of low toxicity to cells, for which reason they are of high safety.

Continued from front page

(72) Inventor: Masao Takahashi
c/o Shunsei Production Company, Ltd.
4-11 Kobuna-cho, Nihonbashi, Chuo-ku
Tokyo-to

(19)日本国特許庁（ＪＰ）　(12)公開特許公報（A）　(11)特許出願公開番号
特開平5－320024
(43)公開日　平成5年(1993)12月3日

(51)Int.Cl.[5]		識別記号	庁内整理番号	FI	技術表示箇所
A61K	7/00	X	9165－4C		
		K	9165－4C		
	7/48		9051－4C		

審査請求　未請求　請求項の数1（全　4　頁）

(21)出願番号　特願平4－154414
(22)出願日　平成4年(1992)5月21日

(71)出願人　000224798
同和鉱業株式会社
東京都千代田区丸の内1丁目8番2号
(72)発明者　鈴木　雅之
東京都千代田区丸の内1丁目8番2号　同和鉱業株式会社内
(72)発明者　重松　一成
東京都千代田区丸の内1丁目8番2号　同和鉱業株式会社内
(72)発明者　陳　玉盤
台湾台北市重慶南路3段116号　心安研究所内
(74)代理人　弁理士　丸岡　政彦

最終頁に続く

(54)【発明の名称】　美白化粧料

(57)【要約】
【目的】　優れた皮膚美白効果を有し、かつ充分な保存安定性および高い安全性を有する美白化粧料の提供。
【構成】　まず、生薬である桑椹の乾燥物約100ｇをミキサーで粉砕し、その粉砕物および1リットルの50%エチルアルコールをフラスコに入れ、撹拌しながら50℃で1時間環流抽出を行う。抽出後、この溶液を吸引濾過し、得られた濾液をエバポレーターを用いて50℃にて減圧濃縮する。次いで、得られた濃縮液を減圧乾燥し、9.2ｇの褐色結晶体（抽出物）を得、得られた抽出物を有効成分として0.01～5.0 %化粧料に配合する。

【特許請求の範囲】

【請求項１】　桑椹、黒豆、蒼耳子および鶏血藤からなる群より選ばれる少なくとも１種の生薬の抽出物を配合したことを特徴とする美白化粧料。

【発明の詳細な説明】

【０００１】

【産業上の利用分野】本発明は、皮膚美白効果を有する美白化粧料に関し、さらに詳しくは、メラニン生成抑制作用に基づく美白効果を有する生薬抽出物を有効成分として配合した美白化粧料に関する。

【０００２】

【従来の技術】一般に、日焼けによる色黒、シミ、ソバカス等は、黒褐色無定形の色素であるメラニンの生成により生じるものと考えられており、このメラニンは、皮膚が紫外線などの外的刺激を受けると、皮膚のメラニン細胞中に存在するチロシナーゼ（チロシン酸化酵素）が活性化し、たんぱく質構成アミノ酸の一種であるチロシンが酸化されて生成する。したがって、メラニン生成に関与するチロシナーゼの活性を抑制することにより肌を白くする効果が期待されるため、チロシナーゼ活性抑制成分の化粧料への配合が提唱されていた。

【０００３】従来、美白効果を有する美白化粧料として、特公昭５５－４３４４３号「美白化粧料」や特公昭５４－９７４号「生薬抽出物配合組成物」に開示されるように、アスコルビン酸またはその誘導体を配合したものが知られている。他にも、アルブチンを配合した皮膚外用剤（特開昭６０－１６９０６号等）やコウジ酸を配合した漂白化粧料（特公昭３２－８１００号）、植物成分（特開昭６３－２９１３号他）または動物成分（特開昭６３－８３１２号他）から抽出した化粧料が美白効果を有するものとして公知である。

【０００４】しかしながら、上記従来の化粧料は、充分な美白効果が認められないものが多く、また、保存安定性が充分でなかったり、刺激性を有するなど皮膚に対する安全性に問題があるものも多かった。

【０００５】

【発明が解決しようとする課題】本発明は、上述従来の技術の問題点を解決し、優れた皮膚美白効果を有し、かつ充分な保存安定性および高い安全性を有する美白化粧料を提供することを目的とする。

【０００６】

【課題を解決するため[illegible]】本発明者等は斯る課題を解決するために鋭意[illegible]桑椹、黒豆、蒼耳子および鶏血藤という[illegible]チロシナーゼ酵素活性阻害作用、および[illegible]におけるメラニン生成抑制作用を有するこ[illegible]、本発明を提供することができた。

【０００７】すなわち、本発明は、桑椹、黒豆、蒼耳子および鶏血藤からなる群より選ばれる少なくとも１種の生薬の抽出物を配合したことを特徴とする美白化粧料を提供するものである。

【０００８】

【作用】本発明の化粧料は、次に示すような方法で製造することができる。まず、桑椹、黒豆、蒼耳子および鶏血藤のうち少なくとも１種の乾燥物または乾燥物の粉砕物を、抽出溶媒を用いて加熱抽出する。なお、該抽出溶媒としては、アルコール（メタノール、エタノール、プロパノールまたはイソプロピルアルコール等）や水などを用いることができ、また、これらの混合溶液を用いることもできる。例えば、アルコール濃度が20～70%の含水アルコールを用い、50℃で１時間の抽出を行うと抽出効率が良い。

【０００９】抽出後、抽出液を濾別して抽出エキスを得る。得られた抽出エキスは、さらに60℃以下の温度で加熱しながら減圧濃縮して乾固させ、乾固した抽出物を回収して化粧料に配合する。なお、上記抽出エキスをそのまま化粧料に配合しても良い。

【００１０】このようにして得た生薬（桑椹、黒豆、蒼耳子、鶏血藤）の抽出物は、従来より用いられてきたアスコルビン酸と比較して、低濃度で優れたメラニン生成抑制作用を発揮することが本発明者等によって確認されており、この抽出物を有効成分として0.01～5.0 %配合することにより、美白効果を有する美白化粧料を得ることができる。

【００１１】以下、実施例により本発明をさらに詳細に説明する。しかし本発明の範囲は以下の実施例により制限されるものではない。

【００１２】

【実施例１】本実施例では、生薬の抽出方法の一例を示す。まず、生薬である桑椹の乾燥物約 100ｇをミキサーで粉砕し、その粉砕物および 500mlの50%エチルアルコールをフラスコに入れ、撹拌しながら50℃で１時間環流抽出を行った。抽出後、この溶液を吸引濾過し、得られた濾液をエバポレーターを用いて50℃にて減圧濃縮した。次いで、得られた濃縮液を減圧乾燥し、 9.2ｇの褐色結晶体（抽出物）を得た。

【００１３】また、上記と同様にして、生薬である黒豆、蒼耳子および鶏血藤の乾燥物約 100ｇから、それぞれ15.6ｇ、 8.8ｇおよび11.9ｇの抽出物を得た。

【００１４】

【実施例２】本実施例では、実施例１で得た桑椹、黒豆、蒼耳子および鶏血藤の抽出物のチロシナーゼ活性阻害作用の測定を行った。なお、チロシナーゼ活性阻害作用の測定は、ドーパからチロシナーゼにより生産されるドーパクロムを、 475nmの吸光度測定によって定量する方法を用いた。また、チロシナーゼ活性阻害作用の測定にあたっては、次のような反応試薬を用いた。

（イ）コハク酸ナトリウムバッファー（100 mM、pH 5.5）

（ロ）マッシュルームチロシナーゼ（SIGMA 社製）溶液

(270 units/mlに（イ）のバッファーで調製)
(ハ) L-DOPA（和光純薬工業（株）社製）溶液（6mMに（イ）のバッファーで調製)
まず、試験管に（イ）のバッファー1.8ml および（ロ）のチロシナーゼ溶液0.1ml を入れ、この試験管に 2%(w/v) 濃度の試料溶液（実施例１で得た抽出物の水溶液）0.1ml を加え、30℃の恒温水槽で15分間インキュベートした。次いで、この試験管に（ハ）のL-DOPA溶液を1ml加え、撹拌した後、30℃の恒温室中で往復振とう機に該試験管を約45° 傾けてセットし、40分間振とう（往復回数150/分）した。振とう後、分光光度計を用いて 475nmの吸光度を測定し、その測定値をＡとした。

【００１５】一方、コントロールとして試料溶液の代わりに（イ）のバッファーを加えたこと以外は上記と同様にして 475nmの吸光度を測定し、その測定値をＢとした。また、ブランクとしてL-DOPA溶液の代わりに（イ）のバッファーを加えたこと以外は上記と同様にして 475nmの吸光度を測定し、その測定値をＣとした。

【００１６】上記 475nmの吸光度の測定値から試料溶液のチロシナーゼ活性阻害率を算出した。なお、チロシナーゼ活性阻害率の算出は、以下の計算式を用いて行い、その結果を表１に示した。

【００１７】チロシナーゼ活性阻害率（%）＝｛1－(Ａ－Ｃ）／Ｂ｝×１００

【００１８】

【表１】

チロシナーゼ活性阻害作用	
生薬抽出物 2%(w/v) 水溶液	チロシナーゼ活性阻害率(%)
桑椹	71
黒豆	72
蒼耳子	69
鶏血藤	96

【００１９】表１からわかるように、桑椹、黒豆、蒼耳子および鶏血藤の[illegible] 2% (w/v) 水溶液という低濃度のものであ[illegible]ーゼの活性を強く阻害しており、優れたチロシナーゼ活性阻害作用を有することが確認された。

【００２０】

【実施例３】本実施例では、実施例１で得た桑椹、黒豆、蒼耳子および鶏血藤の各抽出物のメラニン生成抑制作用の測定を行った。

【００２１】まず、メラニンを生成するマウス由来の悪性黒色腫細胞であるＢ16メラノーマ細胞（B16-F0、ATCC No. CRL-6322 ）を、ウシ胎児血清を終濃度10%となるように添加したイーグルＭＥＭ培地で培養し、６ウェルプレート（FALCON社製）の各ウェルに、該細胞を 3×10^3 cell/ml の濃度で含む上記培地を 6ml入れ、CO_2インキュベーター(5%CO_2、37℃）内で５日間培養した。

【００２２】次いで、この培地を0.03%のテオフィリン(SIGMA社製）を含む新しいイーグルＭＥＭ培地（6 ml）に交換し、各ウェルに適当な量の試料溶液（実施例１で得た抽出物の水溶液）を添加した後さらに３日間培養した。培養終了後、該培地を捨てて各ウェルに１mlの生理食塩水を加え、スクレーパーを用いてウェルの底面に付着している細胞をかきとるように懸濁させた。次に、ピペットを用いて該細胞懸濁液をマイクロ遠心チューブ(1.5ml容量、エッペンドルフ社製）に移し、遠心分離（1,000 ×ｇ、15分間）した。

【００２３】一方、対照として試料溶液の代わりに滅菌水を添加して上記同様の試験を行った。また、細胞の白色化を比較するための実験区として、試料溶液の代わりに 2%Ｌ－アスコルビン酸水溶液を (a)60μｌ、(b)150μｌ、(c)300μｌ添加し、上記同様の試験を行った。

【００２４】次に、ペレットとなった細胞の白色化の度合を目視で比較し、メラニン生成抑制効果の判定を行った。その際、対照実験区（滅菌水添加区）の細胞の白色の度合を「－」、Ｌ－アスコルビン酸を添加した比較実験区の細胞の白色の度合をそれぞれ (a)：「＋」、(b)：「＋＋」、(c)：「＋＋＋」として、試料溶液を添加した場合の細胞の白色の度合が－、＋、＋＋、＋＋＋のどれに対応するかを目視で判断し、試料溶液のメラニン生成抑制効果の強さとして４段階の判定を行った。なお、その結果は表２に示した。

【００２５】

【表２】

メラニン生成抑制作用			
[illegible]度（μg/ml）	50	200	800

桑椹抽出物	＋	＋	＋＋
黒豆抽出物	－	＋	＋
蒼耳子抽出物	＋	＋	＋＋
鶏血藤抽出物	＋	＋	＋＋
濃度（μg/ml）	200	500	1000
Ｌ－アスコルビン酸	＋	＋＋	＋＋＋

【００２６】表２からもわかるように、桑椹、蒼耳子および鶏血藤の各抽出物は50μg/mlの濃度でＬ－アスコルビン酸 200μg/mlと同等のメラニン生成抑制作用を示し、Ｌ－アスコルビン酸よりも低濃度でメラニン生成を抑制することが確認された。また、黒豆の抽出物は 200μg/mlの濃度でＬ－アスコルビン酸と同等のメラニン生成抑制作用を示していた。さらに、各抽出物は 800μg/mlの高濃度であっても細胞に対する毒性はなかった。

【００２７】

【実施例４】本実施例では、実施例１で得た生薬桑椹の抽出物の美白化粧料への配合例を示す。

【００２８】

	(重量%)
桑椹抽出物	1.0
グリセリン	5.0
ポリオキシエチレンソルビタンモノラウレート	1.5
エタノール	10.0
香料	適量
防腐剤、酸化防止剤	適量
色素	適量
精製水	残部

【００２９】

【発明の効果】生薬である桑椹、黒豆、蒼耳子および鶏血藤の抽出物は、優れたチロシナーゼ活性阻害作用、およびメラノーマ細胞におけるメラニン生成抑制作用を有しており、これらの抽出物のうち少なくとも１種を配合した本発明の美白化粧料は、優れた皮膚美白効果を発揮する。また、本発明の美白化粧量に配合される桑椹、黒豆、蒼耳子および鶏血藤の抽出物は、少量で優れた皮膚美白効果を発揮し、細胞への毒性も低いため、安全性の高いものである。

フロントページの続き

(72)発明者　髙橋　雅夫
東京都中央区日本橋小舟町４番11号　順成産業株式会社内